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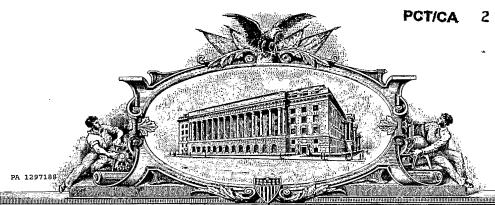
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# PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

	[1	NVENTOR(S	5)			
Given Name (first and middle [if Martin Brent E.				either St oa, Cana		S. PTO )
Additional inventors are being named on the separately numbered sheets attached hereto  TITLE OF THE INVENTION (280 characters max)  NEW PROCESS FOR THE PRODUCTION OF OIL SEED PROTEIN ISOLATES						
Direct all correspondence to:  Customer Number  Customer Number  Customer Number  Direct all correspondence to:  Customer Number  Bar Code Label here  Type Customer Number here						
Address City	Michael I. Stewart  Sim & McBurney  6th Floor, 330 University Avenue  Toronto State Ontario ZIP M5G 1R7					
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Specification Number of Pages 13 CD(s), Number  Drawing(s) Number of Sheets 1 Other (specify)  Application Data Sheet. See 37 CFR 1.76  METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT (check one)  FILING FEE  A check or money order is enclosed to cover the filing fees  The Commissioner is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number fees or credit card. Form PTO-2038 is attached.  The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.  No.  Yes, the name of the U.S. Government agency and the Government contract number are:						
Respectfully submitted, SIGNATURE TYPED or PRINTED NAME Mic	en Du			priate)		:jb

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This collection of information is required by 37 CFR 1.51. The information is used by the public to file (and by the PTO to process) a provisional application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 8 hours to complete, including gathering, preparing, and submitting the complete provisional application to the PTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, Washington, D.C. 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Box Provisional Application, Assistant Commissioner for Patents, Washington, D.C.

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# **Correspondence Information**

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#### TITLE OF INVENTION

# NEW PROCESS FOR THE PRODUCTION OF OIL SEED PROTEIN ISOLATES

#### **FIELD OF INVENTION**

[0001] The present invention relates to the production of oil seed protein isolates, particularly canola protein isolates.

#### **BACKGROUND TO THE INVENTION**

Canola and other oil seed protein isolates having protein contents of at least 100 wt% (N x 6.25) can be formed from oil seed meal by a process as described in copending US Patent Application No. 10/137,391 filed May 3, 2002 (WO 02/089597), assigned to the assignee hereof and the disclosures of which are incorporated herein by reference. The procedure involves a multiple step process comprising extracting canola oil seed meal using a salt solution, separating the resulting aqueous protein solution from residual oil seed meal, increasing the protein concentration of the aqueous solution to at least about 200 g/L while maintaining the ionic strength substantially constant by using a selective membrane technique, diluting the resulting concentrated protein solution into chilled water to cause the formation of protein micelles, settling the protein micelles to form an amorphous, sticky, gelatinous, gluten-like protein micellar mass (PMM), and recovering the protein micellar mass from supernatant having a protein content of at least about 100 wt% (N x 6.25). As used herein, protein content is determined on a dry weight basis. The recovered PMM may be dried.

[0003] In one embodiment of the process, the supernatant from the PMM settling step is processed to recover a protein from the suprenatant. This procedure may be effected by initially concentrating the supernatant using an ultrafiltration membrane and drying the concentrate. The resulting canola protein isolate has a protein content of at least about 90 wt%, preferably at least about 100 wt% (N x 6.25).

[0004] The procedures described in US Patent Application No. 10/137,391 are essentially batch procedures. In copending US Patent Application No. 10/298,678 filed November 19, 2002 (WO 03/043439), assigned to the assignee hereof and the disclosures of which are incorporated herein by reference, there is described a continuous process for making canola protein isolates. In accordance therewith, canola oil seed meal is continuously mixed with a salt solution, the mixture is conveyed through a pipe while extracting protein from the canola oil seed meal to form an aqueous protein

solution, the aqueous protein solution is continuously conveyed through a selective membrane operation to increase the protein content of the aqueous protein solution to at least about 50 g/L, while maintaining the ionic strength substantially constant, the resulting concentrated protein solution is continuously mixed with chilled water to cause the formation of protein micelles, and the protein micelles are continuously permitted to settle while the supernatant is continuously overflowed until the desired amount of PMM has accumulated in the settling vessel. The PMM is recovered from the settling vessel and may be dried. The PMM has a protein content of at least about 90 wt% (N x 6.25), preferably at least about 100 wt%. The overflowed supernatant may be processed to remove canola protein isolate therefrom.

[0005] Canola seed is known to contain about 10 to about 30 wt% proteins and several different protein components have been identified. These proteins include a 12S globulin, known as cruciferin, a 7S protein and a 2S storage protein, known as napin. As described in copending US Patent Application No. 10/413,371 filed April 15, 2003 (WO 03/088760), assigned to the assignee hereof and the disclosures of which are incorporated herein by reference, the procedures described above, involving dilution of concentration aqueous protein solution to form PMM and processing of supernatant to recover additional protein, lead to the recovery of isolates of different protein profiles.

[0006] In this regard, the PMM-derived canola protein isolate has a protein component content of about 60 to about 98 wt% of 7S protein, about 1 to about 15 wt% of 12S protein and 0 to about 25 wt% of 2S protein. The supernatant-derived canola protein isolate has a protein component content of about 60 to about 95 wt% of 2S protein, about 5 to about 40 wt% of 7S protein and 0 to about 5 wt% of 12S protein. Thus, the PMM-derived canola protein isolate is predominantly 7S protein isolate and the supernatant-derived canola protein isolate is predominantly 2S protein. As described in the aforementioned US Patent Application No. 10/413,371, the 2S protein has a molecular size of about 14,000 daltons, the 7S protein has a molecular mass of about 145,000 daltons and the 12S protein has a molecular size of about 290,000 daltons.

#### **SUMMARY OF INVENTION**

[0007] In accordance with the present invention, an alternative procedure is provided to that involving the formation of protein micelles as discussed above, to obtain two canola protein isolates having a protein content of at least about 90 wt%, preferably

at least about 100 wt% (N  $\times$  6.25), one of which is predominantly 7S protein and the other of which is predominantly 2S protein.

[0008] In the present invention, following extraction of protein from the canola oil seed meal, the protein solution is subjected to a first selective membrane step with a membrane having a molecular weight cut-off which permits the 2S protein to pass through the membrane while the 7S and 12S proteins are retained. The retentate then is dried to provide a first canola protein isolate which is predominantly 7S protein. The permeate from the first selective membrane process step is then subjected to a second selective membrane step with a membrane having a molecular weight cut-off which retains the 2S protein and permits low molecular weight contaminants to pass through. The retentate then is dried to provide a second canola protein isolate which is predominantly 2S protein.

# BRIEF DESCRIPTION OF DRAWING

[0009] Figure 1 is a schematic representation of a protein solution recovery process according to one embodiment of the invention superimposed on the protein micelle process.

#### **GENERAL DESCRIPTION OF INVENTION**

[0010] The canola protein isolates provided herein having a protein content of at least about 90 wt% (N x 6.25), preferably at least about 100 wt%, may be isolated from canola oil seed meal by a batch process, or a continuous process, or a semi-continuous process.

[0011] The initial step of the process of providing the canola protein isolates involves solubilizing proteinaceous material from canola oil seed meal. The proteinaceous material recovered from canola seed meal may be the protein naturally occurring in canola seed or other oil seed or the proteinaceous material may be a protein modified by genetic manipulation but possessing characteristic hydrophobic and polar properties of the natural protein. The canola meal may be any canola meal resulting from the removal of canola oil from canola oil seed with varying levels of non-denatured protein, resulting, for example, from hot hexane extraction or cold oil extrusion methods. The removal of canola oil from canola oil seed usually is effected as a separate operation from the protein isolate recovery procedure described herein.

[0012] Protein solubilization is effected most efficiently by using a food grade salt solution since the presence of the salt enhances the removal of soluble protein from the oil seed meal. Where the canola protein isolate is intended for non-food uses, non-food-grade chemicals may be used. The salt usually is sodium chloride, although other salts, such as, potassium chloride, may be used. The salt solution has an ionic strength of at least about 0.10, preferably at least about 0.15, to enable solubilization of significant quantities of protein to be effected. As the ionic strength of the salt solution increases, the degree of solubilization of protein in the oil seed meal initially increases until a maximum value is achieved. Any subsequent increase in ionic strength does not increase the total protein solubilized. The ionic strength of the food grade salt solution which causes maximum protein solubilization varies depending on the salt concerned and the oil seed meal chosen.

[0013] In a batch process, the salt solubilization of the protein is effected at a temperature of at least about 5°C and preferably up to about 35°C, preferably accompanied by agitation to decrease the solubilization time, which is usually about 10 to about 60 minutes. It is preferred to effect the solubilization to extract substantially as much protein from the oil seed meal as is practicable, so as to provide an overall high product yield.

[0014] The lower temperature limit of about 5°C is chosen since solubilization is impractically slow below this temperature while the upper preferred temperature limit of about 35°C is chosen since the process becomes uneconomic at higher temperature levels in a batch mode.

[0015] In a continuous process, the extraction of the protein from the canola oil seed meal is carried out in any manner consistent with effecting a continuous extraction of protein from the canola oil seed meal. In one embodiment, the canola oil seed meal is continuously mixed with a food grade salt solution and the mixture is conveyed through a pipe or conduit having a length and at a flow rate for a residence time sufficient to effect the desired extraction in accordance with the parameters described herein. In such continuous procedure, the salt solubilization step is effected rapidly, in a time of up to about 10 minutes, preferably to effect solubilization to extract substantially as much protein from the canola oil seed meal as is practicable. The solubilization in the

continuous procedure preferably is effected at elevated temperatures, preferably above about 35°C, generally up to about 65°C or more.

[0016] The aqueous food grade salt solution and the canola oil seed meal have a natural pH of about 5 to about 6.8, preferably about 5.3 to about 6.2 are preferred.

[0017] The pH of the salt solution may be adjusted to any desired value within the range of about 5 to about 6.8 for use in the extraction step by the use of any convenient acid, usually hydrochloric acid, or alkali, usually sodium hydroxide, as required.

[0018] The concentration of oil seed meal in the food grade salt solution during the solubilization step may vary widely. Typical concentration values are about 5 to about 15% w/v.

[0019] The protein extraction step with the aqueous salt solution has the additional effect of solubilizing fats which may be present in the canola meal, which then results in the fats being present in the aqueous phase.

[0020] The protein solution resulting from the extraction step generally has a protein concentration of about 5 to about 30 g/L, preferably about 10 to about 25 g/L.

[0021] The aqueous phase resulting from the extraction step then may be separated from the residual canola meal, in any convenient manner, such as by employing vacuum filtration, followed by centrifugation and/or filtration to remove residual meal. The separated residual meal may be dried for disposal.

[0022] Where the canola seed meal contains significant quantities of fat, as described in US Patents Nos. 5,844,086 and 6,005,076, assigned to the assignee hereof and the disclosures of which are incorporated herein by reference, then the defatting steps described therein may be effected on the separated aqueous protein solution and on the concentrated aqueous protein solution discussed below. When the colour improvement step is carried out, such step may be effected after the first defatting step.

[0023] As an alternative to extracting the oil seed meal with an aqueous salt solution, such extraction may be made using water alone, although the utilization of water alone tends to extract less protein from the oil seed meal than the aqueous salt solution. Where such alternative is employed, then the salt, in the concentrations discussed above, may be added to the protein solution after separation from the residual oil seed meal in order to maintain the protein in solution during the concentration step

described below. When a first fat removal step is carried out, the salt generally is added after completion of such operations.

Another alternative procedure is to extract the oil seed meal with the food grade salt solution at a relatively high pH value above about 6.8, generally up to about 9.9. The pH of the food grade salt solution, may be adjusted in pH to the desired alkaline value by the use of any convenient food-grade alkali, such as aqueous sodium hydroxide solution. Alternatively, the oil seed meal may be extracted with the salt solution at a relatively low pH below about pH 5, generally down to about pH 3. Where such alternative is employed, the aqueous phase resulting from the oil seed meal extraction step then is separated from the residual canola meal, in any convenient manner, such as by employing vacuum filtration, followed by centrifugation and/or filtration to remove residual meal. The separated residual meal may be dried for disposal.

[0025] The aqueous protein solution resulting from the high or low pH extraction step then is pH adjusted to the range of about 5 to about 6.8, preferably about 5.3 to about 6.2, as discussed above, prior to further processing as discussed below. Such pH adjustment may be effected using any convenient acid, such as hydrochloric acid, or alkali, such as sodium hydroxide, as appropriate.

[0026] The aqueous protein solution then is concentrated to increase the protein concentration thereof while maintaining the ionic strength thereof substantially constant by a first ultrafiltration step using membranes, such as hollow-fibre membranes or spiral wound membranes, having a molecular weight cut-off sufficient to retain the 7S and 12S proteins and to permit 2S protein to pass through the membrane. A suitable molecular weight cut-off range is from about 30,000 to about 150,000 daltons, preferably about 50,000 to about 100,000 daltons. For continuous operation, the membranes are dimensioned to permit the desired degree of concentration as the aqueous protein solution passes through the membranes.

[0027] The first ultrafiltration step may be effected to concentrate the aqueous protein solution from about 4 to about 20 fold to a protein concentration of at least about 50 g/L, preferably at least about 200 g/L and more preferably at least about 250 g/L.

[0028] The concentrated protein solution preferably then is subjected to a diafiltration step using an aqueous salt solution of the same molality and pH as the extraction solution. An antioxidant may be present in the diafiltration medium during at

least part of the diafiltration step to inhibit oxidation of phenolics in the concentrated canola protein isolate solution. The antioxidant may be any convenient antioxidant, such as sodium sulfite or ascorbic acid. The quantity of antioxidant employed in the diafiltration medium depends on the material employed and may vary from about 0.01 to about 1 wt%, preferably about 0.05 wt%.

[0029] The diafiltration step may be effected by using from about 2 to about 20 volumes of diafiltration solution, preferably about 5 to about 10 volume of diafiltration solution. During the diafiltration operation, phenolics and visible colour components are removed from the concentrated protein solution by passage through the membrane with the permeate. The diafiltration operation may be effected until no significant further quantities of phenolics and visible colour components are present in the permeate.

[0030] The diafiltration step may be effected using the same membrane as used for the concentration step. However, if desired, the diafiltration step may be effected using a separate membrane with a different molecular weight cut-off, such as a membrane having a molecular weight cut-off in the range of about 3000 to about 50,000, preferably about 5000 to about 10,000 daltons, having regard to different membrane materials and configuration.

[0031] The concentration step and the diafiltration step may be effected at any convenient temperature, generally about 20° to about 60°C, preferably below about 30°C, and for a period of time to effect the desired degree of concentration and washing. The temperatures and other conditions used depend to some degree on the membrane equipment used to effect the concentration and the desired protein concentration of the solution.

[0032] The membrane used in the first ultrafiltration step permits a significant proportion of the 2S protein to pass into the permeate, along with other low molecular weight species, including the ionic species of the food grade salt, carbohydrates, phenolics, pigments and anti-nutritional factors. The molecular weight cut-off is normally chosen to ensure retention of a significant proportion of the 7S and 12S protein in the isolate, while permitting the 2S protein and contaminants to pass through having regard to the different membrane materials and configurations.

[0033] The retentate from the concentration step and optional diafiltration step then is dried by any convenient technique, such as spray drying, freeze drying or vacuum

drum drying, to a dry form. The dried protein has a high protein content, in excess of about 90 wt% protein, preferably at least about 100 wt% protein (N x 6.25), and is substantially undenatured (as determined by differential scanning calorimetry). The dried protein isolate consists predominantly of the canola 7S protein, with some 12S protein and possibly small quantities of 2S protein. In general, the dried canola protein isolate contains:

about 60 to about 95 wt % of 7S protein about 2 to about 15 wt% of the 12S protein 0 to about 30 wt% of the 2S protein

[0034] The permeate from the concentration step and optional diafiltration step is concentrated in a second ultrafiltration step using membranes, such as hollow-fibre membranes or spiral wound membranes, having a suitable molecular weight cut-off to retain the 2S protein while permitting low molecular weight species, including salt, phenolics, colour components and anti-nutritional factors, to pass through the membrane. Ultrafiltration membranes having a molecular weight cut-off of about 3,000 to about 30,000 daltons, preferably about 5,000 to about 10,000 daltons, having regard to differing membrane materials and configurations, may be used. The permeate generally is concentrated to a protein concentration of about 100 to about 400 g/L, preferably about 200 to about 300 g/L, prior to drying. Such concentration operation may be carried out in a batch mode or in a continuous operation, as described above for the protein solution concentration step.

[0035] The concentrated permeate may be subjected to a diafiltration step using water. An antioxidant may be present in the diafiltration medium during at least part of the diafiltration step to inhibit oxidation of phenolics in the concentrated permeate. The antioxidant may be any convenient antioxidant, such as sodium sulfite or ascorbic acid. The quantity of antioxidant employed in the diafiltration medium depends on the material employed and may vary from about 0.01 to about 1 wt%, preferably about 0.05 wt%.

[0036] The diafiltration step may be effected using 2 to 20 volumes of diafiltration solution, preferably about 5 to about 10 volumes of diafiltration solution. In the diafiltration operation, further quantities of contaminants, including phenolics and visible colour components, are removed from the concentrated permeate by passage

through the diafiltration membrane. The diafiltration operation may be effected until no significant further quantities of phenolics and visible colour components are removed in the permeate.

[0037] The diafiltration step may be effected using the same membrane as used in the concentration step. Alternatively, a separate membrane maybe used having a molecular weight cut-off in the range of about 3000 to about 50,000 daltons, preferably about 5000 to about 10,000 daltons, having regard to different membrane materials and configurations.

[0038] The concentrated and optionally diafiltered permeate is dried by any convenient technique, such as spray drying, freeze drying or vacuum drum drying, to a dry form. The dried protein has a high protein content, in excess of about 90 wt% protein, preferably at least about 100 wt% (N x 6.25), and is substantially undenatured (as determined by differential scanning calorimetry). The dried canola protein isolate consists predominantly of the canola 2S protein with small quantities of 7S protein. In general, the dried canola protein isolate contains:

about 85 to about 100 wt% of 2S protein 0 to about 15 wt% of 7S protein

[0039] If desired, a portion of the concentrated canola protein isolate from the first ultrafiltration step may be combined with a portion of the concentrated permeate from the second ultrafiltration step prior to drying the combined streams by any convenient technique to provide a combined canola protein isolate composition. The relative proportions of the proteinaceous materials mixed together may be chosen to provide a resulting canola protein isolate composition having a desired profile of 2S/7S/12S proteins. Alternatively, the dried protein isolates may be combined in any desired proportion to provide any desired specific 2S/7S/12S protein profile in the mixtures. The combined canola protein isolate composition has a high protein content, in excess of about 90 wt%, preferably at least about 100 wt% (calculated as N x 6.25), and is substantially undenatured (as determined by differential scanning calorimetry).

[0040] By operating in this manner, a number of canola protein isolates may be recovered as dry mixtures of various proportions by weight of first ultrafiltration-derived canola protein isolate and second ultrafiltration-derived canola protein isolate, generally about 5:95 to about 95:5 by weight, which may be desirable for attaining differing

functional and nutritional properties based on the differing proportions of 2S/7S/12S proteins in the compositions.

#### **EXAMPLES**

# Example 1:

This Example illustrates the process of the invention (Figure 1).

15 kg of canola oil seed meal was added to 100 L (15% w/v) of 0.15M sodium chloride solution at ambient temperature in a 100 L extraction tank and the mixture was agitated for 30 minutes to provide a canola protein solution having a concentration of 20 g/L. Bulk residual meal was removed by using a basket centrifuge with a 400 mesh bag and the separated bulk meal was discharged to waste. The canola protein solution was given a second pass through the basket centrifuge using a 600 mesh bag to remove suspended fine particles. The resulting canola protein solution was polished using a filter press with a 2  $\mu$ m filter pads.

The purified canola protein solution was subjected to an ultrafiltration step using a spiral wound polyvinylidiene difluroide (PVDF) membrane with a molecular weight cut-off of 100,000 daltons at ambient temperature to concentrate the canola protein solution containing 7S and 12S proteins to 4.3 L having a protein concentration of 188 g/L. The permeate from the ultrafiltration step contained the 2S protein along with other low molecular weight species.

[0044] The concentrated canola protein solution (retentate) then was subjected to a diafiltration step using the same membrane as for the ultrafiltration using an aqueous 0.15 M sodium chloride solution containing 0.05 wt% ascorbic acid. The diafiltration medium was added to the retentate at the same flow rate as permeate was removed from the membrane. The diafiltration was carried out with 5 retentate volumes of diafiltration medium.

[0045] A 1.25 L aliquot of the retentate from the ultrafiltration and diafiltration operations was spray dried to provide a canola protein isolate consisting predominantly of 7S protein, having a protein content of 99.1 wt% (N x 6.25, percent nitrogen values were determined using a Leco FP528 Nitrogen Determinator) d.b. and containing 18.21 wt% 2S protein, 74.55 wt% 7S protein and 7.24 wt% 12S protein.

[0046] The permeate from the ultrafiltration and diafiltration operations was subjected to an ultrafiltration step using a spiral wound polyethersulfone (PES)

membrane with a molecular weight cut-off of 5000 daltons to permit retention of 2S protein and to permit low molecular weight contaminants to pass through the membrane to waste. This ultrafiltration step was effected at ambient temperature to concentrate the 2S-containing permeate from the first ultrafiltration step to 3 L having a protein concentration of 125 g/L.

[0047] The concentrated canola 2S protein solution (retentate) then was subjected to a diafiltration step using the same membrane as for the ultrafiltration using filtered tap water. The diafiltration medium was added to the retentate at the same flow rate as permeate was removed from the membrane. The diafiltration was carried out with 5 retentate volumes of diafiltration medium:

[0048] The retentate from the diafiltration step was spray dried to provide a canola protein isolate consisting predominantly of 2S protein, having a protein content of 105.8 wt% (N x 6.25) d.b. and containing 97.0 wt% 2S protein, 3.3 wt% 7S protein and 0.04 wt% of 12S protein.

## Example 2:

[0049] This Example is a report of the process of Example 1, but on a larger scale.

[0050] 150 kg of canola oil seed meal was added to 1000 L (15% w/v) of 0.15 M sodium chloride solution at ambient temperature in a 100 L extraction tank and the mixture was agitated for 30 minutes to provide a canola protein solution having a concentration of 20.7 g/L. Bulk residual meal was removed by using a vacuum filter belt and the separated meal was discharged to waste. The canola protein solution was clarified by using a basket centrifuge and the desludged solids discharged to waste. The resulting canola protein solution was polished using a filter press with 2  $\mu$ m filter pads followed by another one with 0.2  $\mu$ m pads.

[0051] The purified canola protein solution was subjected to an ultrafiltration using two spiral wound PVDF membranes with a molecular weight cut-off of 100,000 daltons to concentrate the canola protein solution containing 7S and 12S proteins for 41.1 L having a protein concentration of 221 g/L. The permeate from the ultrafiltration step contained the 2S protein along with other low molecular weight species.

[0052] A 3L aliquot of the retentate from the ultrafiltration operation was spray dried to provide a canola protein isolate consisting predominantly of 7S protein, having a

protein content of 95.1 wt% (N x 6.25) d.b. and containing 20.21 wt% and 2S protein, 65.75 wt% of 7S protein and 6.92 wt% of 12S protein.

[0053] The permeate from the ultrafiltration operation was subjected to an ultrafiltration step using two spiral wound PVDF membranes with a molecular weight cut-off of 5,000 daltons to permit retention of 2S protein and to permit low molecular weight contaminants to pass through the membrane to waste. This ultrafiltration step was effected at ambient temperature to concentrate the 2S-containing permeate from the first ultrafiltration step to 25L having a protein concentration of 24.2 g/L.

[0054] The retentate from the ultrafiltration step was spray dried to provide a canola protein product having a protein concentration of 47.94 wt% (N x 6.25) d.b. and containing 78.92 wt% of 2S protein along with 15.72 wt% pro-napin protein, 2.06 wt% of 7S protein and 0 wt% of 12S protein.

[0055] The low protein content of the latter canola protein product was due to the absence of a diafiltration step to remove the salt and other impurities. Later bench diafiltration with this product confirmed the production of a canola protein isolate after diafiltration.

## Example 3:

[0056] This Example provides a comparison of the canola protein isolate products prepared according to the procedure of Example 2 with canola protein isolate products prepared by the micelle route.

[0057] A 34 L aliquot of the retentate from the first ultrafiltration step described in Example 2 was warmed to 29.8°C and poured into chilled water having a temperature of 3.7°C at a ratio of 10 volumes of water per volume of retentate. A white cloud of protein micelles immediately formed. The micelles were allowed to coalesce and settle overnight. The accumulated protein micellar mass was separated from supernatant and was spray dried to provide a canola protein isolate having a protein content of 107.4 wt% (N x 6.25) d.b. and containing 3.75 wt% 2S protein, 85.77 wt% 7S protein and 10.32 wt% 12S protein.

[0058] The supernatant from the PMM-settling step (365 L) was subjected to an ultrafiltration step using a spiral wound PVDF membrane with a molecular weight cut-off of 5000 daltons to permit retention of 2S protein and 7S protein and to permit low molecular weight contaminants to pass through the membrane to waste. This

ultrafiltration step was effected at ambient temperature to concentrate the supernatant to 22 L having a protein content of 89.3 g/L.

[0059] The concentrated supernatant was spray dried to provide a canola protein isolate consisting predominantly of 2S protein, having a protein content of 95.51 wt% (N x 6.25) d.b. and containing 84.01 wt% 2S protein and 15.51 wt% 7S protein.

[0060] As may be seen from the above, the process of Example 1 and the micelle process described in this Example produce a canola protein isolate consisting predominantly of 7S protein and having similar protein distributions.

# SUMMARY OF DISCLOSURE

[0061] In summary of this disclosure, canola protein isolates having different protein profiles are obtained using two different ultrafiltration operations employing different molecular weight cut-offs, so that 2S protein present in the permeate from the first ultrafiltration step is purified and recovered in the second ultrafiltration step and 7S and 12S protein is recovered in the retentate of the first ultrafiltration step. Modifications are possible within the scope of the invention.

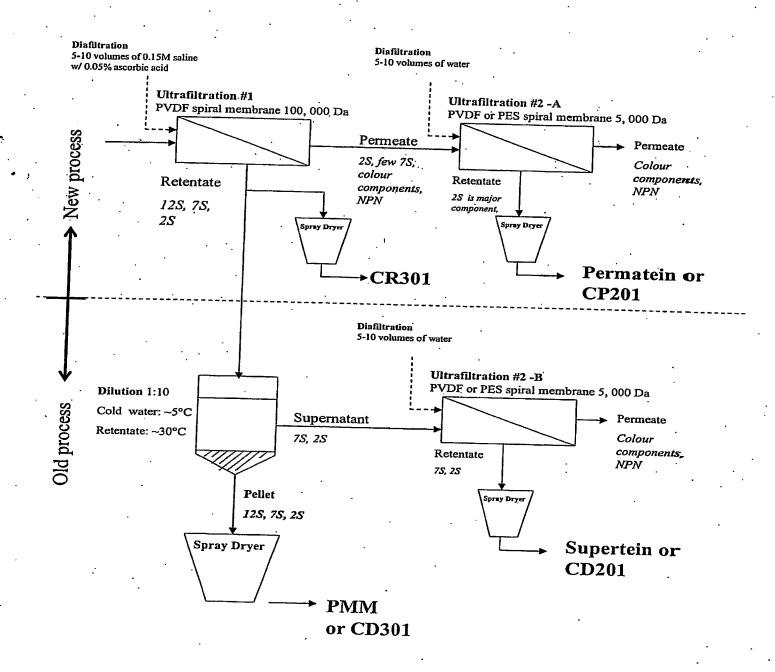


FIGURE 1